Proliferating Cells of Human Basal Cell Carcinoma Are Located on the Periphery of Tumor Nodules

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Study of the growth characteristics of basal cell carcinoma (BCC), a relatively well-organized, slow-growing skin cancer, has been limited because of the lack of methods for propagation of the tumor off the human host. We have used newly developed techniques for transplantation and propagation of BCC on athymic mice to study [^{3}H]thymidine incorporation by nodular BCC. In human BCCs labeled in vitro immediately after removal from the mice and in vivo on the mice, [^{3}H]thymidine during a 4-h pulse was incorporated primarily by cells on the periphery of tumor nodules (labeling indices 6–24%) rather than by the cells more central in tumor nodules (labeling indices 0–2%). Similar results were also seen when samples of tumor freshly removed from patients were labeled in vitro. We conclude that the dividing cells within nodular BCC are primarily the cells at the edges of tumor nodules and that this characteristic is related to the slow, progressive, invasive growth of BCC. *J Invest Dermatol 86:191–194, 1986*

Basal cell carcinomas (BCCs) account for at least 75% of cancer of the skin, the most common site of human cancers, and thus are the most common clinical-histologic subset of human malignancies [1,2]. Although not a cause of significant mortality, BCC does account for considerable morbidity and for significant expenditures of health care dollars.

Study of BCC has been hampered by the difficulties of studying a cancer on the human host, the lack of an animal model, and the inability to propagate the tumor in vitro. In particular, it has not been possible to understand the growth characteristics of this usually well-organized, slowly invasive, but histologically malignant tumor. Previously reported attempts to perform autoradiographic studies of human BCC have not presented data that would localize the proliferating cells to a particular anatomic area of the BCC tumor [3–5].

We have recently described methods by which human BCC may be propagated on the athymic or nude mouse and have shown that BCC growing on the mouse maintains the histology of BCC on the human host [6]. We also noted as part of our pilot studies of 4 tumors that [^{3}H]thymidine uptake by BCC harvested from the nude mouse was not uniform throughout the tumor but was predominantly localized in cells on the periphery of tumor nodules. The primary purpose of this study is to attempt to localize in a large number of tumors by autoradiography following a [^{3}H]thymidine pulse the proliferating cell population within nodular BCC. A second purpose is to demonstrate that the location of the proliferative cell population is similar in BCCs freshly removed from the human host and BCCs on the nude mouse.

MATERIALS AND METHODS

BCC Tissue Tissue was obtained from 10 patients with biopsy-proved nodular BCC who were undergoing Mohs controlled excision. The tumor tissue used for these studies was the portion of the tumor that would ordinarily be discarded in the gross debulking of the tumor prior to stage excision. The BCC tumor tissue was transplanted into splenectomized nude mice who were further immunosuppressed with antilymphocyte serum (MA Bioproducts, Walkerville, Maryland). The details of this technique have been previously described [6]. In 2 cases of large BCCs, a portion of the tumor tissue immediately after removal from the patient was studied by [^{3}H]thymidine uptake and autoradiography.

Tissue Harvesting The growing nodular BCCs on nude mice were removed 60 days after transplantation and were bisected. Half of each specimen was frozen in optimum cutting temperature mounting medium and was sectioned at 4 μm on a cryostat. Sections were stained with hematoxylin and eosin for histologic confirmation of the BCC. The other half was cut into sections 1–2 mm thick, and the sections were incubated with [^{3}H]thymidine.

Pulse Labeling and Autoradiography Portions of the 2 BCCs removed from the patients and the 10 BCCs removed from the nude mice were incubated in vitro with 20 μCi [^{3}H]thymidine (New England Nuclear, NET-027X, 20 Ci/mmol, 1 Ci = 37G Bq) in 5 ml of MCDB 153 medium (Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado). The tissue and the media were gently agitated in a Petri dish on a shaker incubator (Lab-Line Orbit Envivo Shaker, Lab-Line Instruments, Inc., Melrose Park, Illinois) for 4 h at 37°C.

Five nude mice with BCC xenografts were injected i.p. with 50 μCi [^{3}H]thymidine mixed in 0.5 ml of Hanks' balanced salt solution. After 4 h, the mice were sacrificed, and tumor tissue was excised.

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Abbreviations:

BCC: basal cell carcinoma

LI: labeling index

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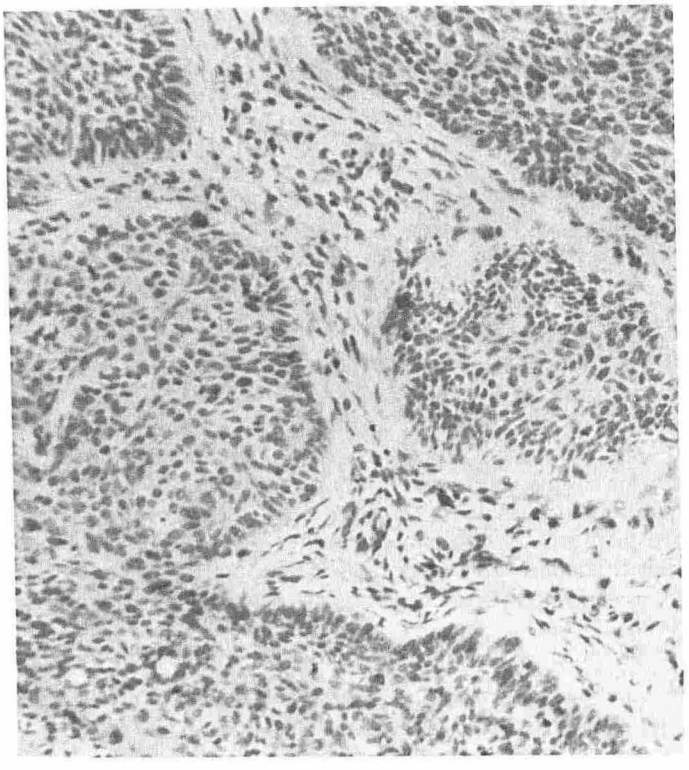

Figure 1. A photomicrograph of a stained section of a BCC after removal from the patient (original magnification × 125). The section demonstrates solid masses of tumor cells and pallisaded nuclei at the edge of tumor masses.

After the 4-h pulse, the BCC tissue from both the in vitro and in vivo labeling experiments was fixed for 4 h in Telly's fixative, rinsed in tap water for 12 h, and embedded in paraffin. The tissue was then sectioned at 5 μm through the center of the tumor nodules, and sections were placed on microscope slides and were deparaffinized. The sections were dipped in a solution of Kodak NBT-2 emulsion, diluted 1:1 with distilled water, and were exposed in light-tight boxes for 7 days at 4°C. The emulsion was developed with Kodak D-19 and fixed with Kodak fixer. The sections were then counterstained with hematoxylin and eosin.

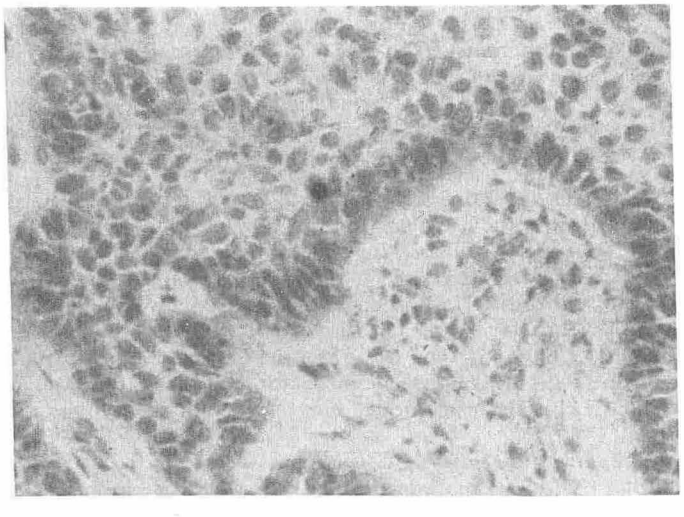

Figure 2. A photomicrograph of a stained section of the same BCC as in Fig 1 after removal from a nude mouse 60 days after transplantation (original magnification × 250). The same histologic features of BCC present prior to transplantation are present after removal from the nude mouse.

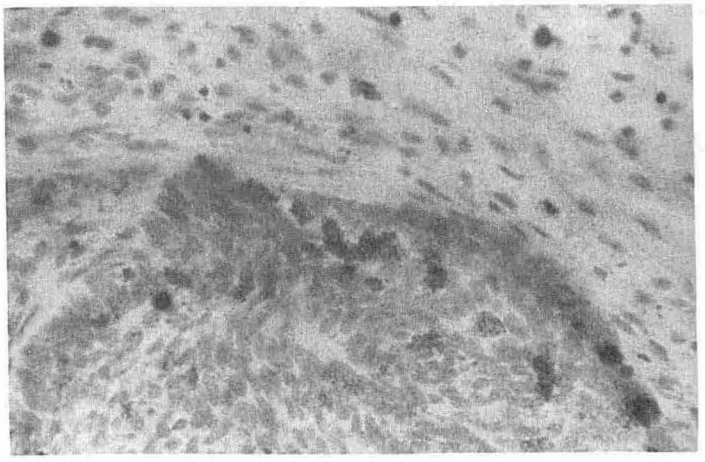

Figure 3. A photomicrograph of a section of a BCC removed from a nude mouse after in-vitro labeling with [^{3}H] thymidine, autoradiography, and staining (original magnification × 250). Labeled nuclei are present predominantly on the periphery of tumor nodules, and few labeled nuclei are present centrally.

Examination of Labeled Sections Labeled cells were defined as well-identified tumor cells with 3 or more silver grains over the nuclei, and with a background of less than 1 grain per cell. For each tumor nodule, a labeling index (LI) was determined for the peripheral cells (outer 5 cell layers) and the central cells. In each zone of a BCC nodule, 200 cells were counted. LIs were calculated as the number of labeled cells per 200 cells.

RESULTS

Light Microscopy of Frozen BCC Specimens The light-microscopic sections of frozen BCC tissue, both immediately after harvest from patients and removal from nude mice, gave good morphologic detail consistent with solid BCC. Fig 1 illustrates the morphology of a solid BCC prior to transplantation into the nude mouse, while Fig 2 depicts the same tumor harvested from the nude mouse 60 days later. Typical histologic features of solid BCC noted include uniform masses of tumor cells with a cell layer on the periphery of the tumor in which nuclei assume a pallisade arrangement. In all 10 tumors, the initial morphology was maintained after transplantation.

Autoradiography Results of autoradiography performed on the 10 BCCs pulse-labeled in vitro after removal from nude mice are illustrated in Fig 3. After a 4-h pulse with [^{3}H]thymidine, labeled nuclei were present predominantly on the periphery of the tumor masses. By far the majority of the cells that took up the label were within the most peripheral cell layer of the tumor, the layer with the pallisaded nuclei. However, labeled nuclei were also noted in cells inside the most peripheral layer. Labeling indexes (Table I) for tumor cells in the outermost 5 cell layers,

Table I. LI of Basal Cell Carcinomas

Tumor	Labeling Method	Number Studied	LI% [mean and (range)] Peripheral Cells	LI% [mean and (range)] Central Cells
BCCs removed from nude mice	In vitro	10	17.4% (11–24)	0.3% (0–1)
	In vivo	5	8.8% (6–13)	1.0% (0–2)
BCCs freshly removed from patients	In vitro	2	11% (10–12)	1.2% (0–2)

including the peripheral pallisaded layer, exceeded those of cells in the central portion of the tumor (17% vs less than 1%).

Similar findings were noted for the 2 tumors labeled in vitro immediately after removal from patients (Fig 4). It is not possible to attach any significance to the small differences in LIs between the tumors harvested from patients and those removed from nude mice (Table I).

In the 5 BCC tumors labeled in vivo in the nude mice, differential labeling between peripheral cells and central cells in the tumor was again seen (Fig 5). The lower LIs for the peripheral cells compared with those obtained in vitro (Table I) may be due to the i.p. administration of [^{3}H]thymidine and the fact that the tumor cells were exposed to a much lower concentration of the labeled nucleotide.

DISCUSSION

Our major conclusions from autoradiographic study of human BCCs harvested from nude mice are that the proliferating cell population within tumor nodules is not uniformly distributed throughout the tumor and that the cells synthesizing DNA are located predominantly on the periphery of the tumors. Although the largest number of labeled nuclei was seen along the most peripheral cell layer with pallisaded nuclei, many labeled nuclei were also seen in the several cell layers inside the most peripheral layer. When the LIs of the outermost 5 cell layers of tumor nodules were compared with those of cells located in the central portions, approximately 10-fold differences were seen. This finding does not negate the possibility that some cells located centrally are capable of DNA synthesis; in fact, a LI of up to 2% was seen for these cells. However, the tremendous differences in LIs that we noted between the 2 zones of BCC nodules would imply that most of the dividing cells are located in the outermost cell layers of the nodules.

With the in-vivo pulse labeling of BCCs, it might be argued that the most peripheral cells in the tumors were exposed to higher concentrations of [^{3}H]thymidine and that this might explain the differences in LIs. However, our finding of even more striking differentials in LIs in BCCs incubated in vitro in [^{3}H]thymidine would suggest that the differences are valid. With the in-vitro labeling of thin sections through tumor nodules, the more peripheral cells and the central cells were exposed to similar concentrations of the labeled nucleotide. We think that these results therefore reflect true differences in the proliferative characteristics of cells in different zones of nodular BCC.

Another major conclusion is that the zonal distribution of proliferating cells noted for BCCs harvested from nude mice reflects a characteristic also seen with tumor immediately after removal from the human host. The BCCs studied immediately after removal from patients gave results quite similar to those seen with BCCs removed from nude mice.

It is difficult to ascertain from previously published studies of human BCC whether a similar zonal distribution of proliferating cells has been noted. Weinstein and Frost [3] injected [^{3}H]thymidine into BCCs on patients and determined the duration of the cell cycle for the tumor cells. As part of this study, LIs after 30- to 60-min pulses were reported to vary between 5–13%. In this study the investigators studied selected, well-labeled small islands of tumor which seemed to show a uniform distribution of labeled nuclei. Specifically excluded were specimens that did not show a uniform distribution of labeled nuclei. Perhaps in this study sections through the peripheral portion of tumor nodules, rather than through the entire tumor nodules, were studied. These investigators considered that BCCs consisted of a relatively homogeneous population of proliferative cells.

In a second study reported by Heenen et al [4] BCCs pulse-labeled in vitro for 15 min produced LIs of 8–10%. These authors state that labeled nuclei were found only in "peripheral zones of tumor fragments," and they therefore restricted their study to the periphery of samples where labeling was uniform. Perhaps these investigators also studied the more peripheral cells of BCC and specifically excluded the more central cells of tumor nodules.

In a third study [^{3}H]thymidine was infused into a patient with BCC for 14 days [5]. The LI rose from 12.5% at 2 h to 80% at

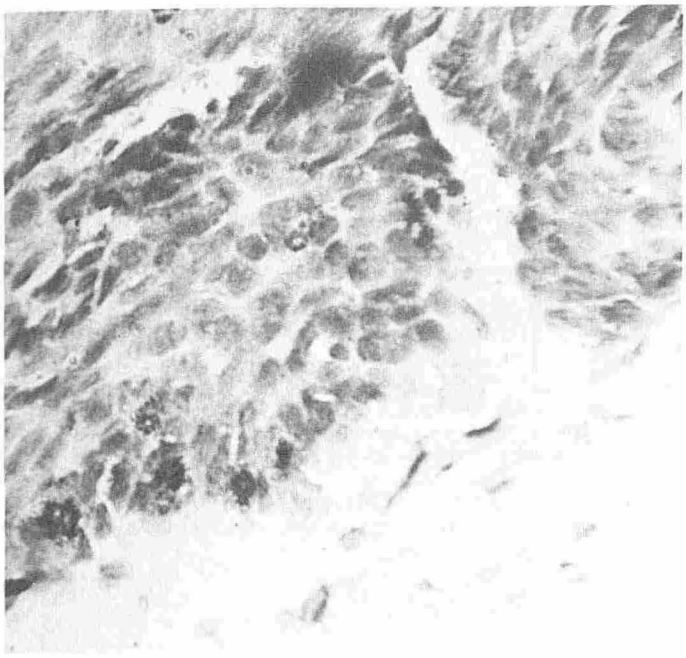

Figure 4. A photomicrograph of a section of a BCC that was labeled in vitro immediately after removal from the patient (original magnification × 250). Labeled nuclei are located largely in the cells at the periphery of tumor nodules. The labeling characteristics of BCCs removed from nude mice (Fig 3) are similar to the characteristics noted for BCCs removed from patients.

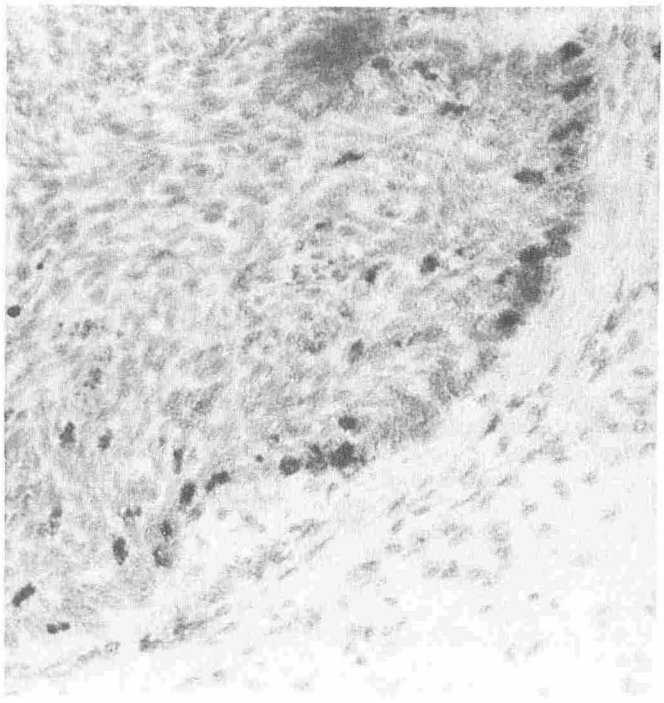

Figure 5. A photomicrograph of a section of BCC labeled in vivo on the nude mouse (original magnification × 250). Most labeled nuclei are present in the outermost cell layers of the BCC.

14 days. No specific details were given regarding where, within the tumor, sections were taken and counts made.

Clinicians have long recognized that BCCs are very slow-growing tumors [1,3] and may require months to years to double in size. This fact makes it difficult to accept that all the cells within a BCC are undergoing cell division at an equal rate, even if the cell cycle is relatively long and similar to that of basal cells of the normal epidermis [3]. Because growth of a tumor represents an excess of cell production over cell loss, the discrepancies between the clinically observed growth characteristics and those reported from experimental studies have been explained by a high rate of cell death in BCC [4]. It is clear that histopathologically regressive changes such as stellar atrophy, pseudo cyst and lacunae formation, and cylindromatous degeneration are seen within BCC [7]. Apoptosis of tumor cells, as evidenced by colloid bodies, and creation of amyloid-like material from remnants of necrotic tumor cells are now well recognized [8–10]. The results of our study would suggest that, in addition to cell death and a relatively long cell cycle, another explanation for the observed slow growth of BCC is that a large portion of the cells within the tumor nodules, specifically the central cells, is not actively proliferating.

The reason for the zonal distribution of proliferating cells within nodular BCC is not apparent. One explanation might be that the proliferative capability of tumor cells in the nodules decreases progressively from the periphery to the center due to physiologic factors, such as a diminished availability of nutrients for more central cells, which are most distant from the stroma and the vascular supply. Another possible exaplanation might be that tumor cells move from the periphery of the tumor nodules to the center and undergo a type of differentiation that is similar to normal epithelial cells. Along with the movement into a "differentiating" compartment of cells would come a diminished potential for division.

Two practical implications of our findings relate to attempts to culture in vitro the cells of BCC and to the therapy of the tumor. We and others have experienced difficulty propagating in tissue culture the cells of a BCC. Part of this difficulty may be due to the fact that a large percentage of the cells of a BCC proliferate slowly or not at all, as indicated by our findings.

Clinicians have long emphasized the necessity of removing all nodular extensions from a BCC for successful surgical therapy of the tumor. If, in fact, these extensions on the periphery of a nodular BCC possess the most actively dividing cells of the tumor, the importance of removal of the peripheral portions of the tumor would be quite understandable.

In conclusion, we think that the ability to propagate human BCC on the nude mouse will facilitate future attempts to better understand the growth of BCC and will allow future studies that are not possible on the human host.

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